Citation:

Bowen J, Noakes M, Clifton PM. Effect of calcium and dairy foods in high protein, energy-restricted diets on weight loss and metabolic parameters in overweight adults. *Int J Obes (Lond)*. 2005 Aug; 29(8): 957-965.

PubMed ID: 15711601

Study Design:

Randomized Controlled Trial

Class:

A - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

POSITIVE: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

- To compare the effect of two isocaloric, energy-restricted high protein diets that differ in dietary calcium and protein source on weight loss and body composition in healthy, overweight adults
- To assess changes in metabolic parameters and risk markers for co-morbidities of obesity following weight loss on high protein diets that differ in protein source.

Inclusion Criteria:

- Men and women
- Body mass index (BMI) of 27 to 40kg/m^2
- Aged 20 to 65 years.

Exclusion Criteria:

- Lactose intolerance
- Widely fluctuating exercise patterns
- Use of calcium supplements
- Use of medications
- Had medical conditions known to affect lipid and glucose metabolism
- Lactating or pregnant.

Description of Study Protocol:

Recruitment

Subject were recruited by public advertisement.

Design

Randomized controlled trial with parallel arm.

Intervention

The study tested the effect of high dairy protein/high-calcium (DP, 2,400mg Ca per day) and high mixed protein/moderate calcium (MP, 500mg Ca per day) diets (5.5mJ per day, 34% protein, 41% carbohydrate, 24% fat) within a 12-week of energy restriction, four weeks of energy balance dietary regime.

Statistical Analysis

- Data analysis was carried out with all subjects who completed the study
- Results are presented for 50 subjects, except dual energy X-ray absorptiometry (DEXA) (N=49 subjects) and urinary analysis (N=47 subjects) due to incomplete data collection. Exclusion of two subjects who did not appear to comply with the dietary protocol (no change in body weight and urinary urea:creatinine) did not affect outcomes or significance
- Data were normally distributed
- An independent T-test was used to assess differences between treatment groups at baseline
- Diet data were analyzed using an unpaired T-test
- The effect of dietary intervention was analyzed using repeated measures ANOVA for an effect of time, diet and gender. Data were re-analyzed with baseline BMI as a covariate.
- Differences were considered significant if $P<0.05$
- Statistical analysis was performed using SPSS v11.0 for WINDOWS.

Data Collection Summary:

Timing of Measurement

- Study consisted of a 12-week energy restriction (ER) phase, followed by a four-week energy balance (EB) phase
- Measurements made at week zero represent baseline data, and week 16 represents the end of the EB phase
- Subjects attended the Clinical Research Unit once every two week for clinical measurements and consultation with a qualified dietitian
- Body weights were measured and overnight fasting (12 hours) venous blood samples were collected in week zero, four, eight, 12 and 16
- In week zero and 16, body composition was assessed
- Subjects attended the clinical research unit on consecutive days to test insulin and glucose responses following a test meal (meal tolerance test; MTT, day one) and glucose load (oral glucose tolerance test; OGTT, day two). Subjects consumed the meal and beverage in 10 minutes. Venous blood samples were collected at baseline then 30, 60, 120 and 180 minutes after commencing the meal and beverage. During these tests, subjects remained at rest and were permitted to sip water
- Urea/creatinine ratio was evaluated in 24-hour urine collected in weeks zero, 12 and 16.

Dependent Variables

- Height was measured on a stadiometer

- Body weight was measured in a regular scale
- BMI was calculated by weight (kg) divided by height $(m)^2$
- Body composition (fat mass of soft tissue and lean mass of soft tissue) was assessed by whole-body DEXA
- Urea and creatinine were measured from 24-hour urine
- Serum insulin was measured using a commercial radioimmunoassay kit
- Serum cholesterol, triacylglycerol (TG) and high-density lipoprotein cholesterol (HDL-C) and plasma glucose were determined using enzymatic kits
- Low-density lipoprotein cholesterol (LDL-C) concentration was determined by a calculation based on the Friedewald equation
- Total area under the glucose and insulin curves (positive incursions only) during MTT and OGTT was calculated using the trapezoidal equation
- C-reactive protein (CRP) was measured using an enzymatic ELISA
- Plasminogen activator inhibitor (PAI), soluble intracellular adhesion molecule (s-ICAM) and vascular cell adhesion molecule (VCAM) were analyzed using commercial ELISA kits. Tissue plasminogen activator (tPA) was measured by enzymatic colorimetric assay
- Total protein (TP), gamma-glutamyltransferase (GGT), bilirubin (BIL), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were measured using enzymatic kits
- Systolic and diastolic blood pressure (SBP and DBP) were measured by automated oscillometry.

Independent Variables

- High dairy protein/high-calcium (DP, 2,400mg Ca per day)
- High mixed protein/moderate calcium (MP, 500mg Ca per day).

Control Variables

- Age
- Baseline values
- Time of observations
- Gender.

Description of Actual Data Sample:

- *Initial N:* 69 subjects
- *Attrition (final N):* 50 (20 males, 30 females)
- *Age:* 25 to 64 years
- *Anthropometrics:* BMI = 25 to 35kg/m^2
- *Location:* Adelaide, South Australia, Australia.

Summary of Results:

- Loss of total weight (-9.7±3.8kg), fat mass (-8.3±0.4kg) and lean mass (-1.6±0.3kg) were independent of dietary group
- There were significant differences in fasting insulin, lipids, SBP/DBP and markers of liver function, fibrinolysis and endothelial function between baseline and 16-week dietary treatment. These differences were independent of dietary intervention with high protein and

calcium, mostly due to caloric restriction.

Body Weight and Body Composition[a] at Week Zero and Change[b] at Week 16

	DP Diet		MP Diet	
	M (N=10)	F (N=15)	M (N-10)	F (N=15)
Body weight (kg)[c]				
Week zero	107.1±3.9	92.8±3.9	101.2±3.4	83.7±2.8
Change at week 16	-9.4±1.3	-9.4±1.0	-12.0±1.5	-7.8±0.6
Lean mass (kg)				
Week zero	62.3±2.7	45.3±2.5	62.2±2.7	45.8±2.8
Change at week 16	-2.1±0.7[d]	-0.9±0.5	-3.3±0.6[d]	-1.0±0.4
Total fat (kg)[c]				
Week zero	41.0±3.0	43.5±2.9[e]	35.2±3.5	36.7±1.1
Change at week 16	-7.5±1.0	-9.2±0.9	-9.6±1.1	-7.1±0.5
Abdominal fat (kg)[c]				
Week 0	10.9±0.7	10.6±0.6[e]	9.2±0.8	8.6±0.3
Change	02.4±0.5	02.8±0.4	-3.0±0.5	-1.9±0.2

DP, dairy protein; MP, mixed protein; change. Differences in body weight and body composition between weeks zero and 16 (after 12-week ER and four-week EB) were analyzed using repeated measures ANOVA; time, factor; diet and gender, between-subject factors.

[a] Determined by DEXA.

[b] All data are mean ±SEM.

[c] Significantly different between week zero and 16, P<0.0001.

[d] Significantly different from females, P=0.004.

[e] Significantly different from females in the MP group, P<0.05.

Other Findings

Fasting Concentration of Lipids, Markers Fibrinolysis, Endothelial Function and Liver Function at Week Zero, Week 16 and Overall Change[a]

	DP Diet (N=25)		MP Diet (N=25)	
	Week Zero	Change Week 16	Week Zero	Change Week 16
Plasma lipids (mmol per L)				
Total-C[b]	5.87±0.24	-0.50±0.15	5.46±0.22	-0.31±0.15
LDL-C[b]	4.17±0.23	-0.49±0.14	3.75±0.21	-0.22±0.14
HDL-C	1.07±0.05	0.07±0.05	1.06±0.05	0.04±0.03

Triacylglycerol[b]	1.38±0.10	-0.18±0.07	1.42±0.11	-0.29±0.10
Fibrinolysis markers				
tPA (IU per ml)	0.61±0.10	0.04±0.09	0.65±0.10	0.26±0.09[c]
PAI-1[b] (AU per ml)	16.0±1.5	-2.6±2.1	18.8±1.7	-6.5±1.8
Endothelial function markers				
s-VCAM (g per L)	2,864±674	-675±677	1,195±151	89±76
s-ICAM[d] (g per L)	416±40	-23±16	36±15	-16±11
CRP[b] (mg per L)	6.5±1.2	-2.4±0.7	5.7±1.0	-1.0±0.4
Liver function markers (U per L)				
GGT[b]	27.2±3.3	-6.3±2.3	35.0±6.6	-12.2±3.1
AST[b]	26.9±2.5	-3.6±2.8	30.9±2.0	-7.3±1.18
TP (g per L)[b]	72.6±0.8	-3.2±0.8	72.6±0.7	-1.3±0.8
Blood pressure (mm Hg)				
Systolic[b]	126±2	-11±2	124±3	-8±2
Diastolic[b]	72±1	-3±1	70±2	-2±1

DP, dairy protein; MP, mixed protein; total-C, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; tPA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor; vWF, von Willebrand factor; s-VCAM, soluble vascular cell adhesion molecule, s-ICAM, soluble intercellular adhesion molecule;CRP, C-reactive protein; GGT, gamma glutamyl transferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; BIL, bilirubin; TP, total protein. Differences were analyzed using repeated measures ANOVA with time as a factor and diet and gender as between-subject factors. By week 16, subjects had completed a 12-ER phase followed by a four-week EB phase.

[a] All data are mean ±SEM.

[b] Significantly different between week zero and 16, independent of diet, $P<0.001$.

[c] Insignificantly greater than DP, $P<0.05$.

[d] Significantly different between week zero and 16, independent of diet, $P<0.05$.

Author Conclusion:

- Weight loss following energy-restricted, high protein diets is not affected by dietary calcium or protein source
- Glycemic control, lipid profile, markers of liver and vascular function and blood pressure improved with weight loss independent of dietary protein source or calcium intake.

Reviewer Comments:

- *This was well-designed and implemented randomized controlled trial with parallel arm design. In this design the baseline values for all the subjects served as control*
- *If all patients were "healthy" obese (without any condition that will make treatment imperative), then a control arm without treatment would have serve for a better control group*
- *A more appropriate design would have been a 2x2 factorial design, as the authors wanted to understand the interaction between protein source and calcium intake on body weight*
- *Intent to treat was not used. Although there were 59 participants that started this study, authors only used complete data from those subjects that finished the study, or 50 patients.*

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions		
1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions		
1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	Yes
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	Yes

2.4.	Were the subjects/patients a representative sample of the relevant population?	Yes
3.	**Were study groups comparable?**	Yes
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	Yes
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	Yes
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	Yes
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	Yes
4.1.	Were follow-up methods described and the same for all groups?	Yes
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	Yes
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	Yes
4.4.	Were reasons for withdrawals similar across groups?	Yes
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	Yes
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	Yes
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A

5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	Yes
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	Yes
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	Yes
7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes

8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	No
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Yes
8.6.	Was clinical significance as well as statistical significance reported?	Yes
8.7.	If negative findings, was a power calculation reported to address type 2 error?	No
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**	Yes
9.1.	Is there a discussion of findings?	Yes
9.2.	Are biases and study limitations identified and discussed?	Yes
10.	**Is bias due to study's funding or sponsorship unlikely?**	Yes
10.1.	Were sources of funding and investigators' affiliations described?	Yes
10.2.	Was the study free from apparent conflict of interest?	Yes